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Predominance and Circulation of Enteric Viruses in the Region of Greater Cairo, Egypt▽

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The circulation of enteric viruses among the population of Cairo, Egypt, between March 2006 and February 2007 was studied. At least one virus was detected in 50% of fecal samples, 57.4% of which were positive for rotavirus, 26% for norovirus, 10.4% for adenovirus, and 1.7% for astrovirus. Over 10% of infections were mixed infections. Rotavirus typing showed that G1P[8] and G2P[4] were predominant but that the unusual G12P[4] and G12P[6] reassortants were also present. Among the noroviruses, half belonged to the predominant GGII.4 cluster. The phylogenetic analysis of the capsid gene suggested that GGII.4 strains from Cairo were similar to those circulating elsewhere. It also showed the emergence of new GGII.4 variants that were not associated with any previously known GGII.4 isolate. Further studies are required to assess the disease burden of enteric viruses in Egypt and the impact of atypical strains.

Acute gastroenteritis is a major cause of morbidity and mortality worldwide (3). Rotaviruses and noroviruses (NoVs) are the most important causative agents of gastroenteritis. Rotavirus infections are responsible for thousands of hospitalizations of children worldwide (27). Additionally, rotavirus is still associated with thousands of deaths in developing countries each year. Epidemiological studies demonstrated that G1 to G4 types and P[4] and P[8] types are responsible for most rotavirus infections, and four G-P combinations, G1P[8], G2P[4], G3P[8], and G4P[8], have been linked to 88.5% of cases of rotavirus diarrhea among children worldwide (13, 30). Other studies have also shown the increasing importance of the G9 rotaviruses, as reviewed previously (10). The G12 rotavirus was first detected in 1990 in the Philippines (35) and has since been found in Asia, Europe, South America, and North America, suggesting that it is possibly emerging all over the world (5, 6, 18, 28, 29, 32). So far, no cases concerning the G12 rotavirus strain have been reported in northern Africa (11, 31).

According to a previous study (15), NoV is the second most common etiologic agent of viral gastroenteritis whatever the age group. To date, five genogroups have been reported for NoV (genogroup I [GGI] to GGV), three of which have been detected from cases of gastroenteritis in humans (GGI, GGII, and GGIV). GGI and GGII are divided into 8 and 19 clusters, respectively (40). For the last 20 years, GGII.4 NoVs have been the predominant NoV genogroup (16). US95/96 isolates have been the most prevalent GGII.4 NoVs associated with gastroenteritis cases worldwide (24). Since 2000, the US95/96 strain has been replaced by new GGII.4 NoV strains, which are characterized by conserved mutations in their capsid and the insertion of one amino acid into the capsid region (9). In 2002, Farmington Hills variants were associated with 80% of NoV-related acute-gastroenteritis outbreaks in the United States (39). The next wave of global epidemics occurred in 2004 and was associated with the emergence of the Hunter variants (4). Early in 2006, the increase in NoV gastroenteritis epidemics was associated with the emergence of two new GII.4 variants, 2006a and 2006b (20, 37). Among the new GGII.4 variants, the Sakai variants were found mainly in Southeast Asia between 2004 and 2006 (26). Phylogenetic analyses showed that each of these GGII.4 variants was directly related to its chronological predecessors, with the exception of the 2006b variant, which is more closely related to the Farmington variants than to the Hunter variants (21, 33). Genetic analysis of GGII.4 NoVs showed that genetic drift and the occurrence of Farmington-like variants have been increasing since 2002. The observation of new isolates was biologically relevant in that patterns of binding to human blood antigens were found to be different from those observed for NoV strains that were isolated before 2002 (21). Overall, the data suggested that new GGII.4 variants appear every 2 or 3 years.

In Egypt, diarrheal illnesses are the major causes of morbidity in children under 3 years of age (23). To date, there have been few epidemiological surveys that aimed to determine the circulation of enteric viruses in Egypt and other countries in the Middle East (11, 31, 36). The aim of this work was to determine the circulation of enteric viruses among the population of Cairo, Egypt. The prevalence of the main enteric-virus infections from 2006 to 2007 was examined. In the second part of the study, we focused on the genetic characterization of the rotaviruses and NoVs detected during the study.

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TABLE 1. Summary of the designed primers used to sequence the GGII.4 NoVs

Primer designation	Sequence	Polarity	Primer location[a]	Amplification region
FW1	5′-GCGATCGCAATCTGGCTCCCAG-3′	+	5055–5076	End of ORF1
FW1-1	5′-CCCCTGGATTAGAAATAATTTTGTAC-3	+	5231–5256	ORF2
RT1-1	5′-ATCAGGGCCCAAGGGCGCGCTCC-3′	−	5314–5336	ORF2
FW2	5′-CTGAAGGCTTGAGCCCCAGCCAG-3′	+	5473–5495	ORF2
RT1	5′-GAGGTTCCAGTTGCCTAACATC-3′	−	5523–5544	ORF2
FW3	5′-GATTCCCCATTCCTTTGGAAAAG-3′	+	5806–5827	ORF2
RT2	5′-GTCGTGCACCTGCCATTTTGTGGTTG-3′	−	5862–5887	ORF2
FW3-1	5′-CAATTATGACCCAACAGAAG-3′	+	6011–6030	ORF2
RT3-1	5′-CCAGTGCTCACTGTAGCTTTGTG-3′	−	6123–6145	ORF2
FW4	5′-CCAAAACACGAAATTCACCCCAG-3′	+	6218–6240	ORF2
RT3	5′-AGGTGYACATTATGACCAGTTC-3′	−	6313–6334	ORF2
FW4-1	5′-AGCGGGTATCCCAACATGAA-3′	+	6405–6424	ORF2
RT4-1	5′-CCTGTRTCTGGATTCACAAATC-3′	−	6508–6529	ORF2
FW5	5′-TGATTTGGTTATCCCCCCCAATGG-3′	+	6596–6619	ORF2
RT4	5′-CGCCCCGTCCCATTTCCCATGG-3′	−	6667–6688	ORF2
FW6	5′-GTCCTTGGCTCTGGACTTGGTTC-3′	+	6740–6762	ORF3
RT5	5′-CTGTTATTTTCAAAYTCAACTTTTG-3′	−	6795–6819	ORF3
FW6-1	5′-CCCGTGGGGCAATCAACGCCCCCATG-3′	+	6993–7018	ORF3
RT6-1	5′-CCAGTACCTTGTTCCGCTCCAGTC-3′	−	7031–7054	ORF3
FW7	5′-TCTAATGCTCCYACTGCYACTTC-3′	+	7181–7203	ORF3
RT6	5′-CTCGTTGAAACAGTTTGATTTG-3′	−	7212–7233	ORF3
RT7	5′-AGATAATCAATTTTGTCTTTTC-3′	−	7514–7535	End of ORF3

[a] The positions of the primers refer to nucleotide positions of MD145 (GenBank accession no. AY032605).

MATERIALS AND METHODS

Sample collection and preparation. Stool specimens were collected at the Tabarak Hospital Cairo and at dispensaries in Gizzeh, Egypt, from 1-month-old to 18-year-old patients who visited the clinics for acute gastroenteritis from March 2006 through February 2007. The patients were routinely checked for symptoms of dehydration. In the event of severe dehydration, the patient was hospitalized for intravenous rehydration until improvement of his status. The stool specimens were routinely tested in Cairo for the presence of bacterial infections and fecal leukocytes. Two hundred thirty clinical specimens from sporadic cases of gastroenteritis for which the bacterium and leukocyte tests were negative were selected for further analysis. During the cold season (September 2006 though February 2007), 73% of these samples ($n = 169$) were collected; the mean age of the patients was 1.7 years, ranging from 1 month to 18 years of age. For the warm season (March through August 2006), 61 samples were collected; the mean age of the patients was 10.6 years, ranging from 1 year to 17 years old. Virological analysis was performed at the National Reference Center for Enteric Viruses in Dijon, France. For each patient, a 10% stool suspension was prepared in phosphate-buffered saline. The stool suspension was used for antigen detection by an enzyme-linked immunosorbent assay. Additionally, 500 to 1,000 μl of the stool suspension was used for the extraction of nucleic acids (DNA and RNA) using a Nuclisens Easy MAG system (bioMérieux, Marcy l'Etoile, France) according to the manufacturer's instructions. RNA was eluted in a final volume of 50 μl and used for NoV detection and typing of the virus-positive samples. The NoV strains E872 (Farmington-like), E1057 (Hunter-like), E1501 (2006a-like), E2703 (2006b-like), and E1267 (2006b-like), which originated from outbreaks of gastroenteritis that occurred in France between 2002 and 2008, were used as reference strains.

Detection and typing of rotaviruses, astroviruses, adenoviruses, and NoVs. The stool specimens were first screened for the presence of group A rotavirus, astrovirus, and adenovirus types 40 and 41 by an enzyme-linked immunosorbent assay, as described previously (31). For the rotavirus-positive samples, the G and P genotypes were determined by reverse transcription-PCR (RT-PCR) using genotype-specific primers, as described previously (12, 14). For rotavirus strains that could not be typed by PCR, the G genotype was determined by sequence analysis of the entire VP7 gene segments, amplified by RT-PCR with primers VP7-F (nucleotides 49 to 71) and VP7-R (nucleotides 914 to 933), as previously reported (17), and primers Beg 9 and End 9 (14). To genotype the adenovirus-positive samples, the primer set Adv-Hex1 DEG/Adv-Hex2 DEG was used to amplify a portion of the hexon gene (1). The nucleotide sequences of the PCR amplicons were determined and compared to those of reference strains. The human astrovirus genotype was determined by sequence analysis of the PCR products obtained with primers Mon244 and Mon245 located in the capsid coding region, as described previously (25).

For NoV detection, the RNA was extracted and purified as described above, and 3 μl was used for each RT-PCR assay. The RT and PCR steps were performed in a single tube by using a Qiagen (Hilden, Germany) one-step RT-PCR kit according to the manufacturer's instructions. The primers JV12 and JV13 were used to amplify a conserved region of the polymerase domain (38). For GGI and GGII NoVs, a conserved region of the ORF2 gene was amplified by using primer sets G1SKF/G1SKR and G2SKF/G2SKR, respectively, as described previously (19). The NoVs detected in the stool samples were genetically characterized by nucleotide sequencing of the PCR products. The genotypes were determined by alignments with reference sequences from GenBank.

Amplification and cloning of the entire sequences of ORF2 and ORF3 of GGII.4 NoVs. Because GGII.4 NoVs were predominant, we determined the entire sequences of ORF2 and ORF3 from these strains for phylogenetic analysis. Three microliters of the extracted RNA was denatured by incubation at 68°C for 10 min and then chilled on ice. The primer pairs FW1/RT5 and FW5/RT7 (Table 1) were used to amplify ORF2 and ORF3, respectively. The denatured RNA and 10 μM of each primer were used in a final volume of 50 μl for RT amplification using a Titan one-tube RT-PCR kit (Roche Applied Biosystems) according to the manufacturer's instructions. RT-PCR was performed

TABLE 2. Age distribution for cases of monoinfection and coinfection among patients with acute gastroenteritis during the study

Type of infection	No. (%) of cases in patients in age group (yr)					
	<1[a]	1–4[a]	5–9	10–14	≥15	Total[b]
Monoinfection						
Rotavirus A	43 [30]	20 [2]	1	2	0	66 (57.4)
NoVs	12 [8]	4 [2]	4	1	2	23 (20)
Adenovirus	5	2	1	2	2	12 (10.4)
Astrovirus	1	0	0	1	0	2 (1.7)
Coinfection						
NoV-rotavirus A	4 [4]	1 [1]	0	0	0	5 (4.3)
NoV-adenovirus	1	1	0	0	0	2 (1.7)
Rotavirus A-adenovirus	1	0	0	0	0	1 (0.9)
Rotavirus A-astrovirus	3 [3]	0	0	0	0	3 (2.6)
NoV-rotavirus A-adenovirus	1 [1]	0	0	0	0	1 (0.9)
Total[c]	71/98 (72.4)	28/60 (46.7)	6/23 (26.1)	6/28 (21.4)	4/21 (19.04)	115 (100)

[a] The number of cases of severe dehydration is indicated in brackets.
[b] The percentage given is the percentage of monoinfections or coinfections per the total number of positive samples.
[c] The value given is the number of positive samples per the total number of samples for each age group.

at 50°C for 60 min; followed by 10 cycles at 94°C for 30 s, 50°C for 30 s, and 68°C for 2 min 40 s; followed by 40 cycles at 94°C for 30 s, 55°C for 30 s, and 68°C for 2 min 40 s, with an increment of 5 s per cycle for the elongation phase; and a final runoff at 68°C for 10 min. The amplicons were analyzed on a 1% agarose gel for the presence of the fragments corresponding to ORF2 and ORF3, respectively. The PCR products were purified from the gel and cloned into the pGEM-T easy vector (Promega France, Charbonnières-les-Bains, France) prior to sequencing.

Nucleotide sequencing and phylogenetic analysis. To genotype NoVs, astroviruses, and adenoviruses, the PCR products were directly sequenced. For the GII.4 NoV strains, the recombinant constructs containing the ORF2 and ORF3 inserts were sequenced in both directions with T7 primer (5′-TAATACGACTCACTATAGGG-3′) and SP6 primer (5′-TATTTAGGTGACACTATAG-3′), in addition to the newly designed primers listed in Table 1. The sequencing reaction was performed by using an ABI Prism big dye terminator cycle sequencing ready reaction kit v1.1 (Applera, France) and an ABI 3100 automated sequencer (PE Biosystems). The nucleotide sequences were edited with the CodonCode Aligner program version 2.0.4 (Dedham, MA). The sequences were aligned using Clustal W2 (Heidelberg, Germany).

A set of 67 (ORF2) and 37 (ORF3) representative strains from GenBank and this study was used to construct phylogenetic trees based upon amino acid sequences. The alignment of the sequences was generated using Clustal W from the MEGA 4.0 package (34). The percentage of identity between isolates or clusters was calculated using MEGA 4.0 software. The percentage of identity was determined according to the number of nucleotide changes per site. The consensus trees were each generated from 1,000 replicates using the neighbor-joining (NJ) method for clustering (MEGA 4.0 software). To study evolution between strains, a minimum spanning tree (MST) from the Bionumerics package (Applied Maths BVBA, Sint-Martems-Latem, Belgium) was constructed using the default setting. The MST was calculated according to amino acid changes, and unlike the NJ method, the MST takes into account the position of each variation. Of note, for this type of tree, it is assumed that each amino acid change is unique and that reverse mutations do not occur. The MST was based upon 193 complete ORF2 amino acid sequences of GGII.4 from GenBank and this study (the list is available upon request). Version 3.5.1 of Simplot software was used to detect putative recombination events between ORF2 and ORF3. The ORF2 and ORF3 plasmid constructions for the Cairo strains are available upon request.

Nucleotide sequence accession numbers. The NoV nucleotide sequences reported here have been deposited in GenBank under accession numbers EU876882 (Cairo 2), EU876883 (Cairo 3), EU876884 (Cairo 4), EU876885 (Cairo 5), EU876886 (Cairo 6), EU876887 (Cairo 7), EU876888 (Cairo 8), EU876889 (Cairo 9), EU876890 (E1057 Dijon), EU876891 (E2703 Dijon),

TABLE 3. Distribution of group A rotavirus G and P genotypes among patients with acute gastroenteritis

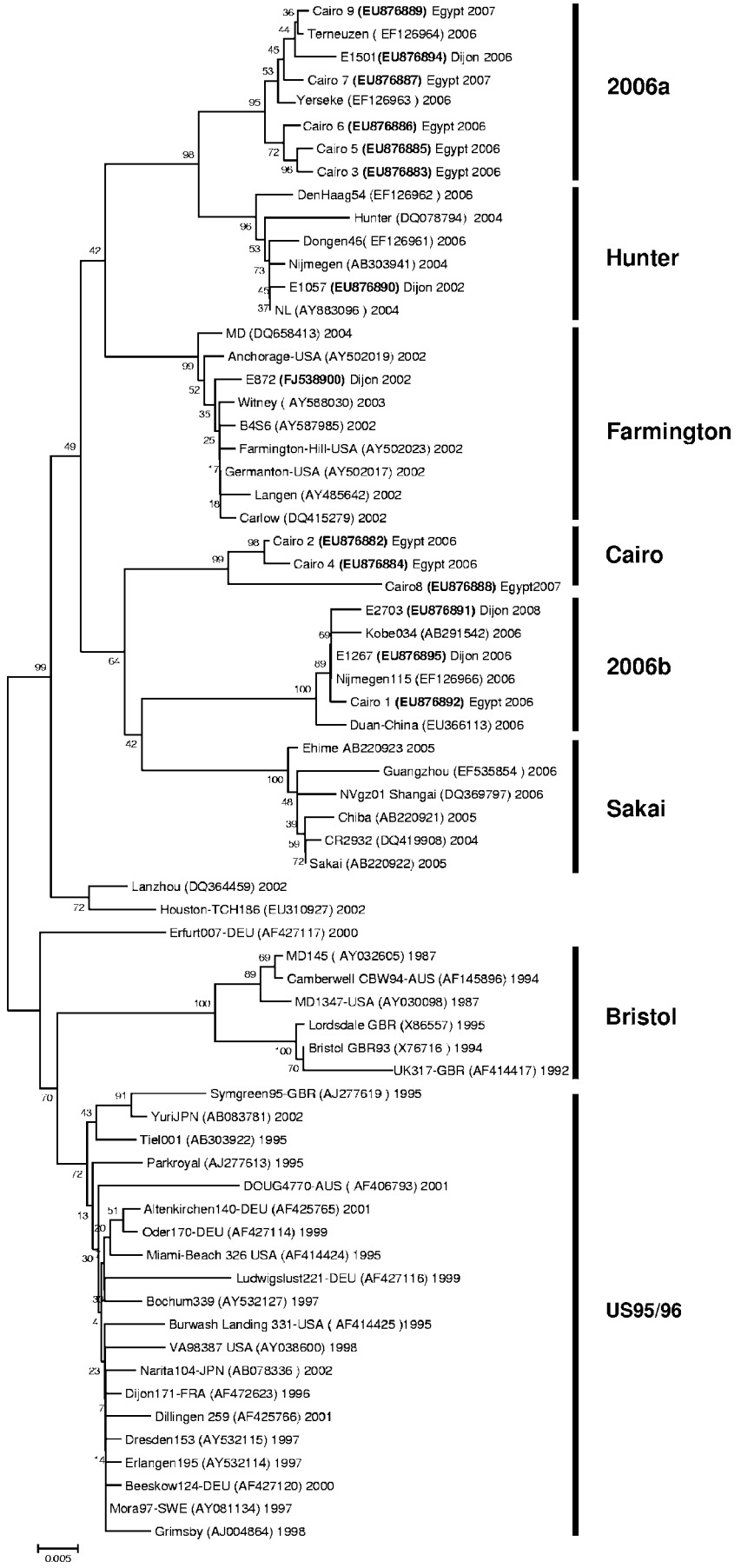
Genotype	No. of samples				
	P[8]	P[4]	P[6]	Mixed[a]	Total
G1	13	0	2	0	15
G2	5	31	1	4	41
G9	6	1	0	0	7
G12	0	1	6	0	7
NT[b]	4	1	0	1	6
Total	28	34	9	5	76

[a] For G2-related mixed infections, P[4] and P[8] were detected in three samples. P[4] and P[6] were detected in one sample. For the nontypeable rotavirus for the G genotype, P[4] and P[8] were detected in the specimen.
[b] NT, nontypeable.

TABLE 4. Distribution of NoV genotypes from patients with acute gastroenteritis

No. (%) of NoV-positive specimens	Genotype (isolate[s]) or total no. (%) of specimens[a]	
	Polymerase	Capsid
8 (25.8)[b]	GGII.4 (Cairo 2 through 9)	GGII.4 (Cairo 2 through 9)
4 (12.9)[b]	GGII.4 (Cairo 10)	ND
1 (3.2)[b]	ND	GGII.4 (Cairo 1)
1 (3.2)	GGII.4	GGII.15
4 (12.9)[c]	GGIIb	GGII.3
3 (9.7)	GGIIb	ND
1 (3.2)	GGII.3	ND
3 (9.7)	ND	GGI.1
3 (9.7)	ND	GGI.9
1 (3.2)	ND	GGI.3
1 (3.2)	ND	GGI.4
1 (3.2)	ND	GGI.5
31 (100)	21 (67.7)	23 (74.2)

[a] The primer set used for detection in the polymerase region was JV12/JV13, and the primer sets used for detection in the capsid region were G2SKF/G2SKR and G1SKF/G1SKR. ND, not detected.
[b] For the Cairo 2 through Cairo 9 isolates, both the ORF2 and ORF3 nucleotide sequences were determined. For the Cairo 1 and Cairo 10 isolates, the ORF2 and ORF3 sequences, respectively, were determined.
[c] For one sample, the sequencing of the junction confirmed the presence of recombinant GGII2b-GGII.3, as described previously (2).


Cairo 9 (EU876889) Egypt 2007
Terneuzen (EF126964) 2006
E1501(EU876894) Dijon 2006
Cairo 7 (EU876887) Egypt 2007
Yerseke (EF126963) 2006
Cairo 6 (EU876886) Egypt 2006
Cairo 5 (EU876885) Egypt 2006
Cairo 3 (EU876883) Egypt 2006
2006a
DenHaag54 (EF126962) 2006
Hunter (DQ078794) 2004
Dongen46(EF126961) 2006
Nijmegen (AB303941) 2004
E1057 (EU876890) Dijon 2002
NL (AY883096) 2004
Hunter
MD (DQ658413) 2004
Anchorage-USA (AY502019) 2002
E872 (FJ538900) Dijon 2002
Witney (AY588030) 2003
B4S6 (AY587985) 2002
Farmington-Hill-USA (AY502023) 2002
Germanton-USA (AY502017) 2002
Langen (AY485642) 2002
Carlow (DQ415279) 2002
Farmington
Cairo 2 (EU876882) Egypt 2006
Cairo 4 (EU876884) Egypt 2006
Cairo8 (EU876888) Egypt2007
Cairo
E2703 (EU876891) Dijon 2008
Kobe034 (AB291542) 2006
E1267 (EU876895) Dijon 2006
Nijmegen115 (EF126966) 2006
Cairo 1 (EU876892) Egypt 2006
Duan-China (EU366113) 2006
2006b
Ehime AB220923 2005
Guangzhou (EF535854) 2006
NVgz01 Shangai (DQ369797) 2006
Chiba (AB220921) 2005
CR2932 (DQ419908) 2004
Sakai (AB220922) 2005
Sakai
Lanzhou (DQ364459) 2002
Houston-TCH186 (EU310927) 2002
Erfurt007-DEU (AF427117) 2000
MD145 (AY032605) 1987
Camberwell CBW94-AUS (AF145896) 1994
MD1347-USA (AY030098) 1987
Lordsdale GBR (X86557) 1995
Bristol GBR93 (X76716) 1994
UK317-GBR (AF414417) 1992
Bristol
Symgreen95-GBR (AJ277619) 1995
YuriJPN (AB083781) 2002
Tiel001 (AB303922) 1995
Parkroyal (AJ277613) 1995
DOUG4770-AUS (AF406793) 2001
Altenkirchen140-DEU (AF425765) 2001
Oder170-DEU (AF427114) 1999
Miami-Beach 326 USA (AF414424) 1995
Ludwigslust221-DEU (AF427116) 1999
Bochum339 (AY532127) 1997
Burwash Landing 331-USA (AF414425)1995
VA98387 USA (AY038600) 1998
Narita104-JPN (AB078336) 2002
Dijon171-FRA (AF472623) 1996
Dillingen 259 (AF425766) 2001
Dresden153 (AY532115) 1997
Erlangen195 (AY532114) 1997
Beeskow124-DEU (AF427120) 2000
Mora97-SWE (AY081134) 1997
Grimsby (AJ004864) 1998
US95/96
0.005

EU876892 (Cairo 1), EU876893 (Cairo 10), EU876894 (E1501 Dijon), EU876895 (E1627 Dijon), and FJ538900 (E872 Dijon).

RESULTS

Sample collection and virus detection. Two hundred thirty stool specimens from cases of gastroenteritis were selected for further investigation for enteric viruses based upon the absence of pathogenic bacteria and fecal leukocytes. Eighty percent ($n = 184$) of the 230 patients presented mild ($n = 133$) or severe ($n = 51$) dehydration. Half of the 230 cases were positive for at least one enteric virus (Table 2), and were all associated with mild ($n = 64$) or severe ($n = 51$) dehydration. The bulk of the positive specimens were detected during the cold season (88.7%, $n = 102$). Thirteen of the 61 tested specimens were positive for enteric viruses during the warm season. Ten percent of the viral infections were mixed viral infections. Sixty and 86% of the positive samples were taken from patients younger than 1 and 5 years of age, respectively. For patients over the age of 5 years, only 22% of cases of gastroenteritis were viral infections, and no mixed infections were observed. Rotaviruses and NoVs were the predominant viruses detected during the survey and accounted for 87.8% of viral infections, including mixed infections. The adenovirus was the third cause of viral diarrhea in our study and was detected in all age groups. Human astrovirus was detected in five patients; three of these were mixed infections. All cases of severe dehydration ($n = 51$) were associated with either rotavirus ($n = 32$) or NoV ($n = 10$) monoinfections or mixed infections (rotavirus-NoV [$n = 5$], rotavirus-astrovirus [$n = 3$], or rotavirus-NoV-adenovirus [$n = 1$]). Severe dehydration occurred mostly in children under 1 year old.

Typing. For group A rotaviruses, the P and G genotypes were determined by multiplex RT-PCR and sequencing (Table 3). The P genotype was determined for all strains. Three P genotypes were found, P[4], P[8], and P[6], which accounted for 51.3, 42.1, and 13.2% of the rotavirus strains (including the mixed infection), respectively. Four G genotypes were detected. G2 and G1 genotypes predominated and accounted for 53.9 and 19.7% of the rotavirus strains, respectively. G9 and the rare G12 genotypes were also detected, and each accounted for 9.2% of rotavirus strains. The G genotype could not be determined for six rotavirus strains. The rotavirus strains G2P[4] and G1P[8] were the most predominant and accounted for 40.8 and 17.1% of rotavirus strains, respectively. All but one G9 strain showed a P[8] genotype, and the G12 rotaviruses were associated mainly with P[6]. All of the G9- and G12-related strains were detected in children under the age of 5 years. The G12 rotavirus isolates were all associated with severe dehydration. Three rotavirus strains, which were detected in 8-, 11-, and 12-year-old patients, were P typed but were not typeable for the G type. These data suggested that these G types might be unusual variants infecting older children and could not be amplified by the typing primers that we used.

NoVs accounted for 27% of the enteric viruses based upon our PCR results. Twenty-nine percent and 71% of the strains belonged to NoV GGI and GGII, respectively (Table 4). Among the GGII NoVs, GGII.4 strains predominated, representing 45% of all of the NoV isolates. For one sample, GGII.4 and GGII.15 were detected in the polymerase and the capsid regions, respectively, which might suggest the presence of both NoVs in the stool specimen. The newly characterized GGIIb recombinant was also detected often ($n = 7$) and was associated with the GGII.3 capsid genotype for four samples. Sequencing the ORF1-ORF2 junction of one isolate confirmed that the GGIIb NoV in the polymerase was indeed GGII.3 in the capsid, as described previously (2). For the GGI NoVs, GGI.1 and GGI.9 accounted for two-thirds of the GGI NoV strains. Of note, the percentage of GGI NoVs could have been underestimated during the study since they were detected with only one set of primers.

Phylogenetic analysis of GGII.4 NoVs. For several years, GGII.4 NoVs have been the most predominant NoVs detected in Europe and North America during outbreaks of gastroenteritis (8, 20). To determine whether similar GGII.4 NoVs were circulating in Egypt, the entire nucleotide sequences of ORF2 were determined for the GGII.4 isolates that were found in Cairo. Given the similarity between the sequences in the capsids of the 2006a and 2006b strains from this study and those of the GGII.4 NoVs isolated in Europe (e.g., France), it can be suggested that similar strains circulate among Mediterranean countries (Fig. 1). Moreover, we detected three new GGII.4 NoV strains, belonging to none of the known variants. The tree constructed from the amino acid sequences using either the NJ (Fig. 1) or the maximum-parsimony (data not shown) method clearly showed that the three new variants clustered together to form a new subgroup, the Cairo cluster. For all but the Bristol and the Cairo variants, we observed at least 95% nucleotide identity within each cluster (Table 5). The percentage of nucleotide identity between subgroups ranged from 88.4% (Bristol and 2006b) to 96.1% (Hunter and 2006a). The average amino acid identity between variants was 94.5% and ranged from 92.1% (Bristol and Sakai) to 97.7% (Hunter and 2006a) (Table 5). Previous studies showed the increasing diversity of NoVs within GGII.4 strains (4, 20, 21, 22, 33). To determine the minimum number of amino acid changes between strains, an MST was constructed based upon the complete amino acid sequences of ORF2 using a set of 193 sequences corresponding to the complete ORF2 of Bristol, US95/96, Farmington, Hunter, Sakai, 2006a, 2006b, and Cairo variants (Fig. 2). The MST is an alternative tool to study the relationship between closely related NoV strains. It is important to mention that a complete set of sequences, includ-

FIG. 1. Phylogenetic tree based upon the amino acid sequences of ORF2 from 67 representative strains from GenBank and GGII.4 isolates from this study. The groups of variants and outlier strains are indicated to the right of the tree. Bootstrap values are percentages of 1,000 iterations. The scale is indicated at the bottom and corresponds to the number of amino acid substitutions per amino acid residue. The GenBank accession numbers of the strains are indicated in parentheses. The NoV isolates sequenced during this study are indicated in bold.

TABLE 5. Pairwise comparison of nucleotides and amino acids based upon the complete ORF2 sequences of the GGII.4 NoVs from GenBank and from this study

NoV	Nucleotide or amino acid identity (%)[a]							
	Bristol (n = 6)	US95/96 (n = 69)	Farmington (n = 69)	Hunter (n = 13)	2006a (n = 8)	2006b (n = 10)	Sakai (n = 11)	Cairo (n = 3)
Bristol	92.9–99.7	(95.8)	(93.3)	(92.5)	(92.6)	(92.2)	(92.1)	(92.6)
US95/96	92.4	95.5–100	(95.5)	(94.9)	(94.5)	(94)	(94.4)	(94.6)
Farmington	89.9	93.8	95.2–100	(96.1)	(96.1)	(95.4)	(94.9)	(94.3)
Hunter	89.4	93	94	97.2–100	(97.7)	(95.1)	(95.4)	(94.4)
2006a	89.4	92.8	94.1	96.1	98–99.9	(94.5)	(94.4)	(94.3)
2006b	88.4	92	92.7	92.2	91.8	98.1–99.8	(95.3)	(95.1)
Sakai	89.2	93.1	94.1	93	92.9	92.6	98.4–99.3	(94.9)
Cairo	88.9	91.9	90.6	90.2	90.4	90.1	90.5	91.7–98.8

[a] n indicates the number of strains for each subgroup. The Lanzhou (DQ364459), Houston TCH-186 (EU310927), Ast6139 (AJ583672), and Erfurt (AF427117) isolates were not included in the analysis. Amino acid identity values are given in parentheses. A range of values indicates the maximum and minimum nucleotide identities within each group of variants.

ing those of outlying strains (e.g., Erfurt [Fig. 2]), must be used to construct a valid MST. Overall, the lineages and variants found for the MST matched those observed for the NJ tree (Fig. 1). The MST was built with 484 amino acid changes. The MST suggested that 2006a and 2006b might be directly related to the former Hunter and Farmington variants, respectively, as previously described (33). The tree showed that the Hunter, Farmington, and Sakai variants might all be related to the US95/96 variants via the Lanzhou isolate, which might be considered an intermediate strain. The MST showed that the three strains belonging to the Cairo group were distant from the Sakai variants (data not shown) and the US95/96 (Fig. 2) isolates by the same number of amino acid changes (n = 23).

In the last part of the study, we analyzed the complete GGII.4 ORF3 sequences to determine whether ORF3 pre-

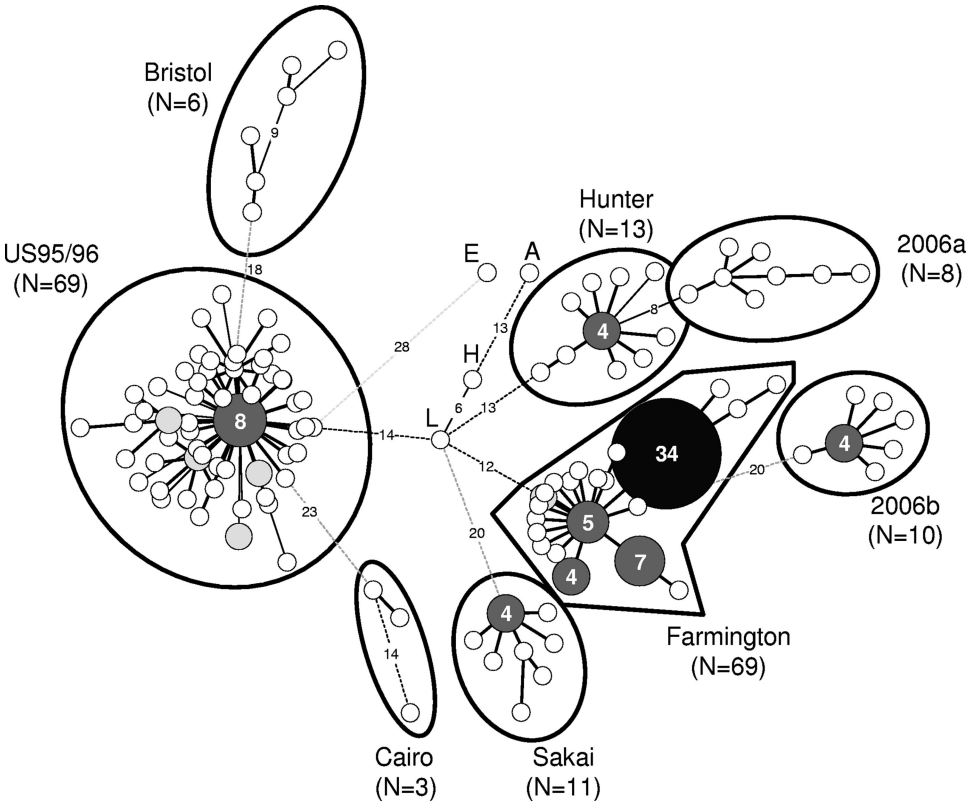


FIG. 2. MST based upon the alignment of 193 complete amino acid sequences of ORF2 of GGII.4 NoVs from GenBank and this study. The numbers of amino acid changes between strains are indicated on the connecting lines. The groups of variants have arbitrarily been defined by six or fewer amino acid changes. The number of sequences for each group of variants is indicated in parentheses. Identical sequences are represented by color-coded circles, which are scaled according to member count. The white and light-gray circles represent one and two sequences, respectively. For dark-gray and black circles, the number of identical sequences is indicated inside each circle. The isolates Erfurt, Lanzhou, Houston, and Ast6139 are indicated by the initials E, L, H, and A, respectively.

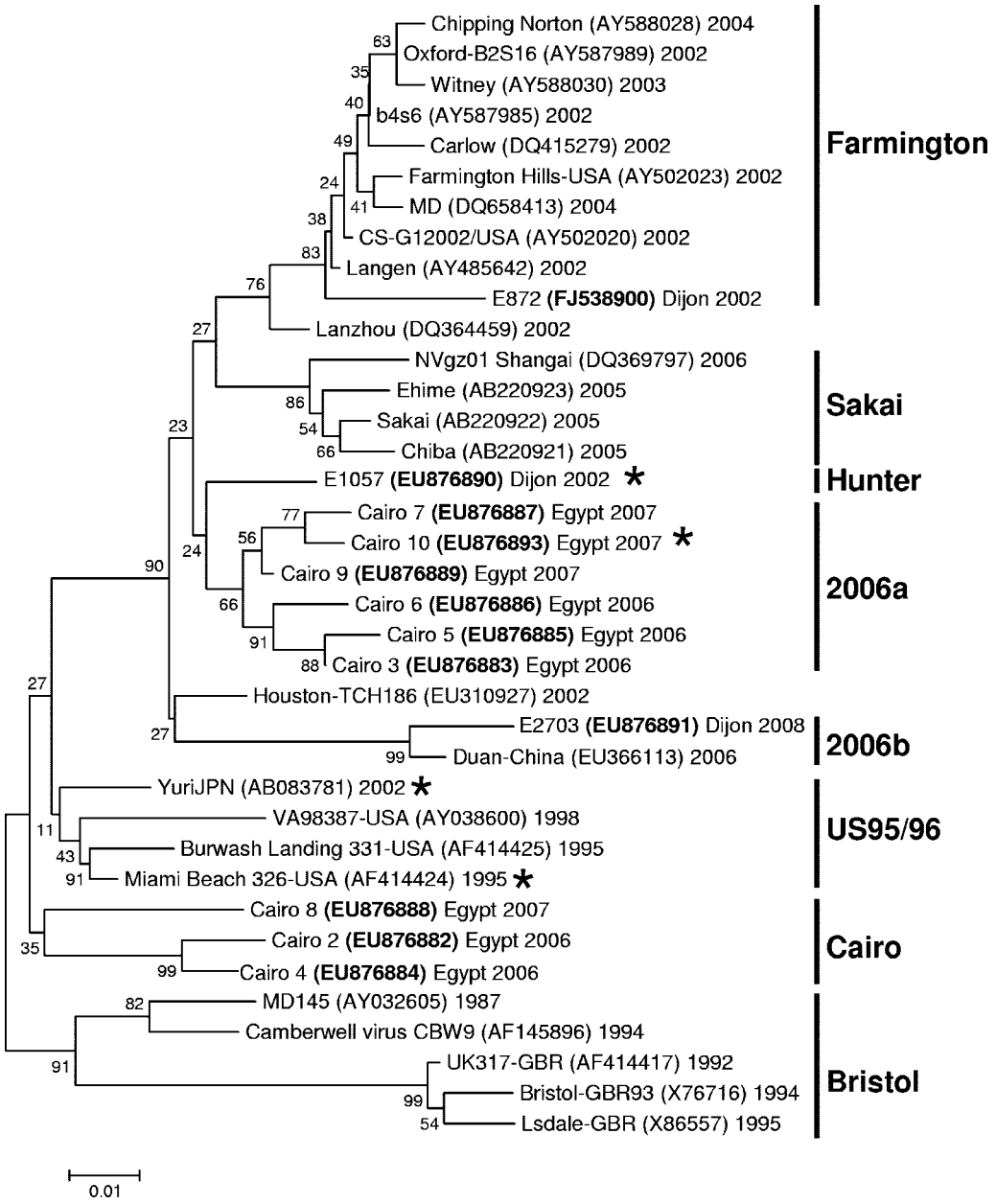


FIG. 3. Phylogenetic tree based upon the amino acid sequences of ORF3 from 37 strains from GenBank and GGII.4 isolates from this study. The variants are indicated to the right of the tree. Strains with deleted amino acids in ORF3 are indicated by an asterisk. The bootstrap values and scale are described in the legend for Fig. 1. The GenBank accession numbers of the strains are indicated in parentheses. The NoV isolates sequenced during this study are indicated in bold.

sented the same genetic distribution as ORF2. For the E1057, Cairo 10, Miami Beach, and Yuri strains, we observed the deletions of 1 (position 145), 2 (positions 145 and 146), 6 (positions 206, 207, and 209 through 212), and 7 (positions 150 through 153 and 155 through 157) amino acid residues, respectively, located in the variable region of ORF3, as described previously (7). For E1057 and Cairo 10, the presence of the deletions was confirmed by direct sequencing of the PCR products from the stool specimens (data not shown). Despite the less available sequence information for ORF3, 37 representative sequences from this study and GenBank were used to construct a phylogenetic tree. We observed clustering of the variants similar to that previously observed for ORF2 (Fig. 3). However, the Cairo variants were related to the US95/96 and Bristol variants in ORF3, while they were closely related to 2006b in ORF2 (Table 5 and Fig. 1). Additionally, the Lanzhou and Houston strains did not cluster together in ORF3. The variations between ORF2 and ORF3 that we observed might suggest an intragenotypic recombination event(s) between groups of variants, which may have been impossible to detect because of the strong homology between isolates (data not shown).

DISCUSSION

Although the disease burden of enteric viruses has been well characterized for Western countries, there is little information about these viruses in the Middle East. In this study, we report on the surveillance of enteric viruses from 230 cases of gastroenteritis that occurred in the Cairo agglomeration. Fifty percent of the patients were infected by at least one virus, and severe dehydration was observed for 51 patients. Common strains of rotaviruses and NoVs were detected during this study, but so were unusual variants, like G12 rotaviruses and a new variant of GGII.4 NoVs (Cairo).

Rotaviruses and NoVs were the main causative agents of viral gastroenteritis in Cairo, as was recently described for Tunisia (11, 31). Our study showed that G1P[8] and G2P[4] rotaviruses accounted for 17% and 40.8% of rotavirus infections, respectively. The ratio between G1P[8] and G2P[4] is rather unusual since G1P[8] is usually predominant. However, a study conducted in rural Egypt in 1995 and 1996 showed that G2P[4] accounted for 67% of the rotavirus-positive samples (23). It is also noteworthy that G12 was the third most common G type detected in our study. Prior to this study, this serotype had not been detected in Egypt. Since the first G12 rotaviruses were isolated in the Philippines in 1990 (35), the number of G12 strains reported worldwide has been increasing, suggesting their possible emergence.

The typing of the NoVs from this study showed that GGI and GGII strains are circulating in the Cairo agglomeration. During the past decade, most NoV-related outbreaks have been associated with the emergence of novel GGII.4 strains, all of which presented an inserted amino acid in the P2 domain of the capsid (9, 21, 33). The sequence analysis of ORF2 of the Egyptian GGII.4 isolates and the GenBank strains clearly demonstrated that NoVs found in Egypt were similar to those circulating in Europe since 2006 and were mostly related to the 2006a and 2006b variants. Interestingly, three of the Egyptian GGII.4 strains (Cairo variants) were not associated with any GGII.4 cluster that has previously been described. The MST analysis showed that Farmington, Sakai, and Hunter strains were all related to US95/96. Our data also suggest that the Cairo strains might be directly related to the US95/96 isolates or the Sakai variants. Additional sequencing data are required to determine whether the Cairo strains are linked to either group. The detection of these new variants might indicate the increasing epidemiological importance of the Cairo isolates locally (e.g., in Egypt and the Middle East) or worldwide. Further studies are required to determine whether the Cairo variants might replace the predominant 2006a and 2006b variants.

Little is known about the function of the VP2 protein, and despite a limited number of available sequences, our data clearly showed the presence of the same variants as described for ORF2. It is noteworthy that the insertion of one amino acid in the P2 domain of ORF2 has been a common feature among the new GGII.4 strains isolated since 2002. Inversely, the deletion of amino acids in the ORF3 region was not found in all GGII.4 isolates and probably did not give a selective advantage. The topologies of the ORF2 and ORF3 trees showed different organizations of the variants, suggesting that the mechanisms by which ORF3 is evolving might be different from those described for ORF2 (22, 33).

The finding that a wide variety of enteric viruses are circulating among the population of Cairo raises questions about emerging viral pathogens. Indeed, we observed an unusual rotavirus distribution, with G2P[4] being the most predominant rotavirus, and the presence of newly discovered G12 rotavirus reassortants. We also characterized new GGII.4 NoV variants (named the Cairo group). Continuing surveys are required to determine whether these new variants will become predominant in the Middle East and elsewhere in the world. Additionally, the fact that 51 of the patients with viral gastroenteritis had severe dehydration requiring hospitalization emphasizes the need for better prevention of these viral infections. The occurrence of new enteric viruses also raises questions about the efficacy of existing and future vaccines for the large population of Cairo. Finally, the data that we present here emphasize the need for further surveys of circulating enteric viruses in the Middle East, where heavily populated agglomerations like Cairo may favor the emergence of unusual strains.

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